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WM. A. TAYLOR, Chief

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MORPHOLOGY OF THE BARLEY GRAIN WITH REFERENCE TO ITS ENZYM-SECRETING AREAS

By

ALBERT MANN, Plant Morphologist, and H. V. HARLAN,
Agronomist in Charge of Barley Investigations

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By ALBERT MANN, *Plant Morphologist, Office of Agricultural Technology*, and H. V. HARLAN, *Agronomist in Charge of Barley Investigations, Office of Cereal Investigations.*

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INTRODUCTION.

The value of the barley crop to the American farmer depends upon two factors, the yield per acre and the price per bushel. An increase of revenue is as readily effected by one as by the other. The yield is necessarily an agricultural problem; the price is also, within certain limits. Although the daily price of any market product ordinarily varies over a considerable range, higher values are placed upon those offerings which most perfectly meet the requirements of consumers. A superior quality is the equivalent of a greater quantity. The nearer a farmer can come to producing a product ideally suited to its uses, the higher will be the price which he will be able to command.

By far the greatest demand upon the barley crop is for the purpose of malting. This operation consists essentially in the breaking down of the cell walls of the endosperm of the barley grain so as to leave its starch grains exposed to later enzymatic actions, and also in the abundant production of these enzyms, both the diastatic and proteolytic. The abundant formation of diastase has long been con-

NOTE.—This paper is intended for distribution to agronomists, station directors, brewery chemists, and selected maltsters.

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sidered to be one of the most important functions of the malting process. In small-berried malts the excess of diastase is often used in brewing to convert quantities of inert starch in addition to that found in the grain itself. In some large-berried malts it may be so used; in others, it is best used in conjunction with the other ferments to convert a large endosperm and thereby obtain a high percentage of extract.

The possibility of this improvement in quality was the cause of the special study made of this grain. Early in the investigation it was realized that the desirability of any barley must rest largely on its morphology, because the physiological changes must owe their origin to morphological sources. An extensive study of the barley grain, both at rest and in germination, was outlined. It was later found that the investigation had to be extended to include the embryology of the grain, in order to explain certain features of its resting condition. The Office of Foreign Seed and Plant Introduction, where a large part of these investigations was carried on, was of great service in obtaining for study samples of barleys from almost every country of the world. The primitive barleys of Asia and the most specialized productions of Europe were compared in structure and in the details of germination. A persistent agreement between malting quality and morphological structure was found throughout the observations, and the conclusions pointed toward a hopeful method for securing a better quality in this grain.

A brief report which discussed the secretion of diastase and cytase in the barley grain was made by the senior writer[1] in 1908. Such statements in this paper as relate to the histological phase of secretion are also based upon his studies.

STRUCTURE OF THE BARLEY GRAIN.

A ripened grain of barley is a very complex structure. It contains not only the usual essential members of growth, but certain adhering layers of tissue which remain from organs functional at earlier periods of development. The gross anatomy of the grain may be separated into three divisions: The seed proper contains the young plantlet and the food stored for its use in germination. Enveloping the seed is a covering of several layers which, because it includes the remains of both the ovary walls and the integuments, has been given the name of caryopsis. In most of the cereals this caryopsis is freed from the glumes or floral leaves by thrashing. With barley this is not usually the case. In all but a few forms the glumes are grown fast to the caryopsis, and the grain presents the appearance shown in figure 1. While the relation of the parts is more apparent when considered in the order enumerated, a study of the structure more logic-

[1] Mann, Albert. A new basis for barley valuation and improvement. U. S. Department of Agriculture, Bureau of Plant Industry Circular 16, 8 p., 3 fig., 1908.

ally proceeds from the glumes inward. The glumes, the seed coat of the caryopsis, and the seed proper, each consists of several kinds of tissue.

THE GLUMES.

The outer surface of the glume is protected by a heavy cuticle. The cuticularization does not penetrate deeper than the first row of cells. The three outer layers of cells have heavily reinforced (sclerenchymal) walls, while the underlying layers are thin walled and more variable. They are usually three or four in number. The vascular bundles of the glume are normal leaf bundles

THE SEED COAT OF THE CARYOPSIS.

The term "seed coat of the caryopsis" is here used to include groups of tissues from three separate origins: The pericarp, the testa, and the semipermeable membrane. The pericarp is the remains of the ovary wall. Its outer layer lies flat against the glume and is united with it. Beneath the layer in contact with the glume are several layers of parenchyma, consisting of much flattened and often almost disconnected cells. Below this are two layers of parenchyma that formerly contained chlorophyll. Though equally flattened through pressure, they are in much better condition as a layer. The cells of this tissue are almost at right angles to those of the main parenchyma region and tangential to the grain. On the inside of the pericarp are sometimes found scattering fragments of the inner epidermis of the ovary, their cells also elongated lengthwise with the grain.

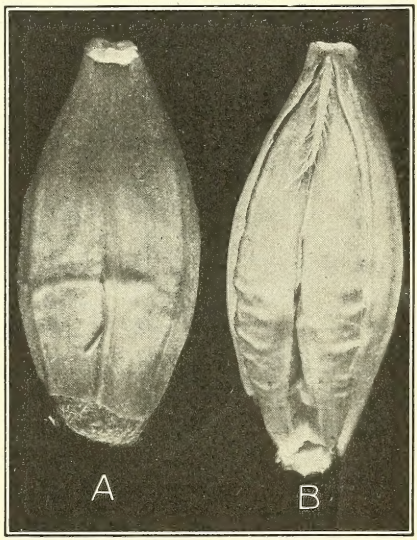


FIG. 1.—A grain of 2-rowed barley: *A*, Dorsal view; *B*, ventral view.

The testa is the remnant of the inner of the two integuments that once existed inside the ovary wall, the outer one having disappeared. The cells of the testa are crushed almost to the point of disappearance, being represented in the ripened grain by a mere line. In intimate contact with the testa and much better preserved is the investing membrane of the nucellus. It consists of moderately thickened cells flattened by pressure. On the inside of this are occasional patches of partly reabsorbed cell walls, remnants of the nucellar tissue. According to Brown,[1] the investing membrane of the nucellus probably forms the semipermeable membrane, though the inner integument may also be concerned.

[1] Brown, A. J. On the existence of a semipermeable membrane inclosing the seeds of some of the Gramineæ. Annals of Botany, v. 21, no. 81, p. 79–87, 1907.

THE SEED PROPER.

The external layer of the seed proper is the aleurone layer. It assumes a greater importance in barley than in other grains. It normally consists of a stratum of two or three rows of cubical cells gorged with protein contents. Their appearance shows them to be cells of unusual vigor and vitality. The greater part of the space within the aleurone layer is occupied by the starch endosperm. This is, as in all grasses, a typical storage tissue. Its cells are thin walled and packed with starch granules. The nuclei are feeble and distorted by the pressure of the accumulated starch. At the proximal end of the starch endosperm and partly embedded in its tissue lies the embryo, the morphology of which is observed with ease but interpreted with difficulty. From its nature, it must contain all the growing elements of the plant to be, as well as a number of modified organs. The embryo lies obliquely to the long axis of the grain, its growing point directed toward the distal end. The true vegetative point is inclosed within the first leaves of the plant. In barley these are twisted, giving rise to the term "acrospire" in place of "plumule." The plumule sheath surrounds all the upper vegetative portions. At the proximal end of the embryo is found a single main radicle. A short distance above, possibly at the first rudimentary internode, are five to eight secondary rootlets. When eight are present three are placed on either side and two toward the front. The space between the secondary rootlets and the growing point serves as a base for the attachment of the extra-embryonic tissue, the scutellum, or shield (fig. 2), which includes practically all the remaining tissue. The origin and significance of this body as well as of the epiblast (absent in this genus, but common in grasses) has been variously interpreted. The scutellum is, however, generally conceded to be an absorbing organ. It is made up of thin-walled cells with large nuclei. Its inner surface rests directly upon the mass of the endosperm, and this surface of contact consists of a layer of elongated cells placed endwise to both the endosperm and the rest of the scutellar body. This layer, which is known as the epithelial layer, plays, according to the observations of the writers, a large part in the chemical activities of germination.

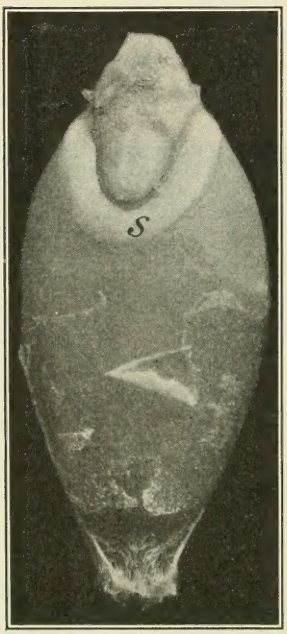
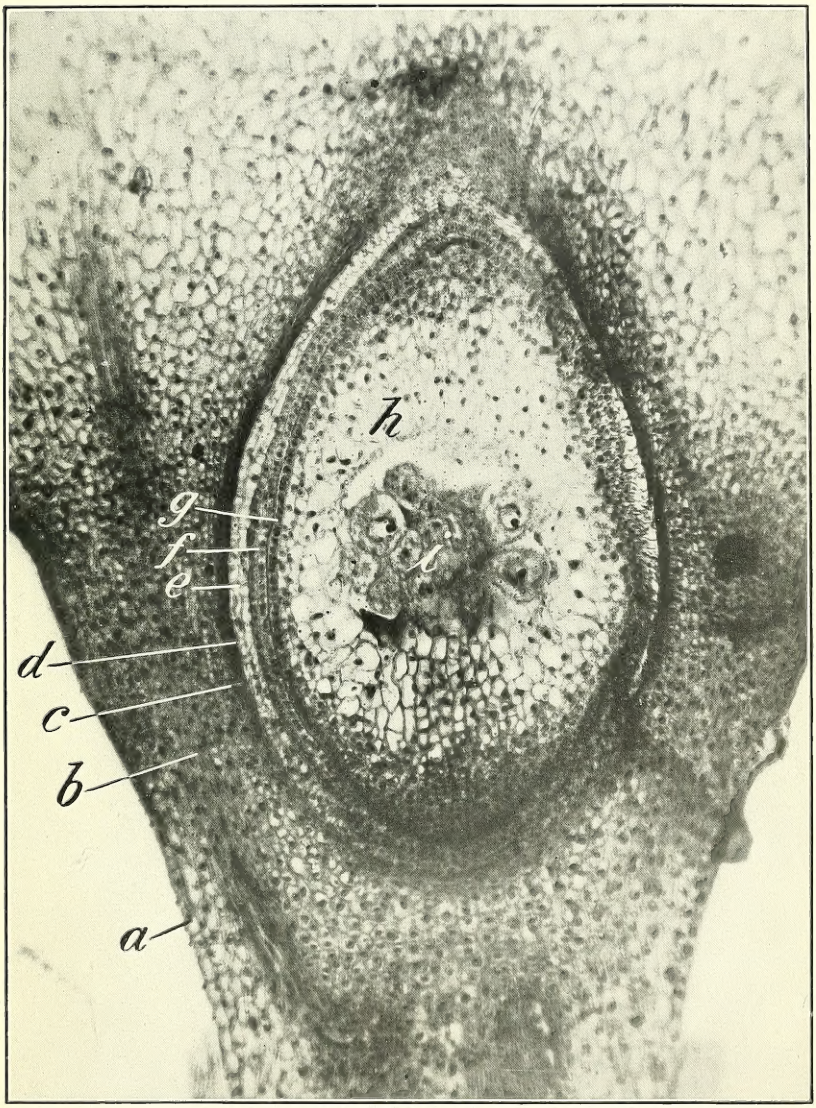


FIG. 2.—A high-grade barley grain with the glumes removed to show the embryo with its collarlike scutellum (*s*). The inner envelopes have been removed from the upper part of the grain.



PHOTOMICROGRAPH OF A LONGITUDINAL SECTION OF A BARLEY OVARY IMMEDIATELY AFTER FERTILIZATION.

The two plumose stigmas do not appear. *a*. Outer epidermis of ovary; *b*, colorless parenchyma; *c*, chlorophyll-bearing parenchyma; *d*, inner epidermis of ovary; *e*, outer integument of ovule; *f*, inner integument of ovule; *g*, external layer of the nucellus; *h*, nucellar tissue; *i*, group of endosperm-forming cells of the young embryo sac.

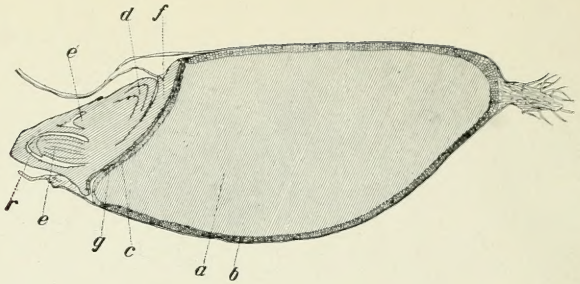


FIG. 1.—LONGITUDINAL (DORSI-VENTRAL) SECTION OF A MATURE BARLEY GRAIN.

a, Starch endosperm; *b*, aleurone layer; *c*, area of starch endosperm, adjacent to the scutellum, representing the initial stage of the corrosion of the endosperm. This took place at the close of the ripening of the grain. *d–r*, The embryo; *d*, plumule with investing sheath; *e*, primary, and *e'*, secondary, radicles; *f*, scutellum, the surface next to the endosperm covered with the epithelial layer (*g*); *r*, root cap of primary radicle.

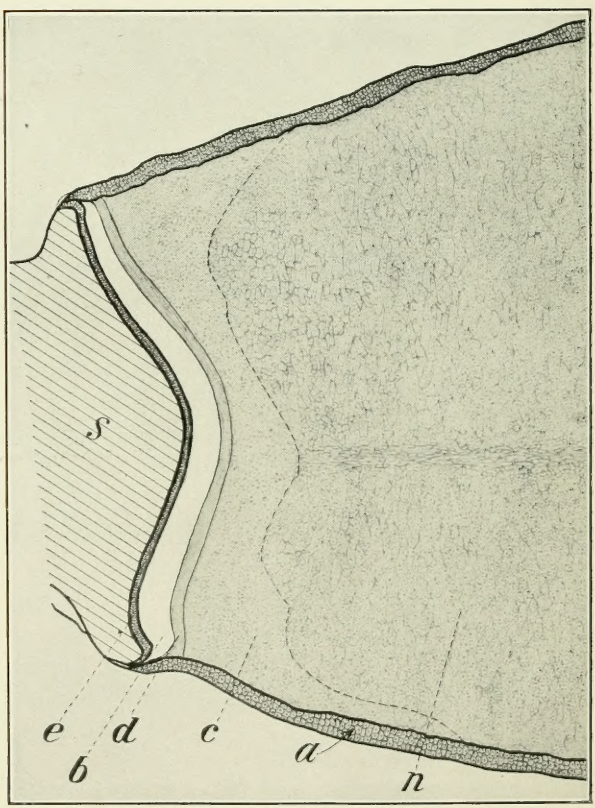


FIG. 2.—LONGITUDINAL SECTION OF BARLEY GRAIN, SHOWING THE STAGE OF ENDOSPERM CONVERSION AT THE END OF THE SECOND DAY OF GERMINATION.

The starch endosperm is being broken down in the dense areas directly in front of the epithelial layer, (*e*), of the scutellum (*s*), and more rapidly in the less dense areas adjacent to the aleurone layer, (*a*). *b*, Clear fluid area produced during germination; *c*, corroded area of starch endosperm; *n*, normal starch endosperm not yet attacked.

DEVELOPMENT OF THE BARLEY GRAIN.

An understanding of the office and behavior of the various organs of the barley grain is greatly facilitated by observations upon their origin and development. Some of these organs are functional throughout the existence of the seed; others important in the early stages afterwards disappear, while still others become so modified as to be serviceable in a totally different way.

The flower of the barley plant is inclosed within the flowering glumes, as in all grasses. It consists of a simple ovary containing a single ovule. The stigma is two branched and plumose. Three versatile anthers attached below the ovary fit into as many natural angles in the glumes. On the dorsal side of the ovary are two lodicules.

At the time of flowering, the ovary wall is a but slightly modified vegetative structure. It consists of the usual epidermis, a colorless parenchyma of several layers, a chlorophyll-bearing parenchyma, and an inner epidermis. The chlorophyll layer is the only one in any way exceptional. The cells of this lie with their long axes at right angles to that of the others and tangential to the grain. Inside the ovary are two integuments which probably act as conductive tissues to the growing pollen tube during fertilization. Each is formed of two layers of thin-walled cells. The cells of the outer integument are somewhat larger than those of the inner one and are much thinner walled. The contrast between the two integuments is very evident in Plate I. Inclosed by the two integuments is the nucellus, which is in turn surrounded by its own investing membrane, consisting of a single layer of cells. In the parenchyma mass of the nucellus is the embryo sac, which before fertilization contains the usual eight cells. At this stage of development the cells of all the tissues are filled with plasma.

After fertilization the ovary increases in length and width. As the essential parts of the developing seed are taking shape, certain now useless portions of the enveloping structures become modified and in some cases absorbed. The plasma gradually disappears from the cells of the outer integument, the cell walls become transparent, and finally dissolve and disappear. The inner epidermis is also absorbed, although not always completely, as traces sometimes persist at maturity. A little later, portions of the ovary wall begin to weaken. The starting point is usually just outside the chlorophyll layer, and the process advances outward through the overlying parenchyma layers. At maturity this tissue is represented by a compressed mass of almost disconnected cells. In the earlier stages of growth it is this reduction of tissue that establishes the "green ripeness" of Kudelka, the removal of the overlying cells bringing the chlorophyll layer nearer to the surface, thus intensifying the

green color. The chlorophyll-bearing cells themselves become somewhat thickened and remain plainly discernible as a layer, even at maturity. The inner integument does not disappear as does the outer one. The cell walls undergo considerable modification and the layer becomes crushed, so that it exists in the ripened fruit only as a dull brown line. It is in the investing membrane of the nucellus, however, that the greatest functional transformation occurs. The cell walls of this delicate tissue become thickened, and the layer quickly assumes, either independently or in conjunction with the inner integument, the functions of a selective semipermeable membrane. Brenchley states that even before the glumes are grown fast to the fruit it is necessary to prick through this membrane to bring about the entrance of killing fluids. The absorption of the nucellar tissue begins almost immediately after fertilization has taken place. It proceeds rapidly and is almost total, a small body persisting near the funicle and fragments of collapsed cell walls in chance locations elsewhere.

Of the tissues which envelop the seed, the semipermeable membrane is the only one performing more than mechanical service. On the other hand, all the growth within the embryo sac is of physiological purpose in one or more ways. There are three important structures—the aleurone layer, the embryo, and the starch endosperm (Pl. II, fig. 1). The three are associated in development and are intimately related in later germination and growth. Preliminary to fertilization, the nucleus of the embryo sac divides and redivides until eight nuclei are present. The egg cell and two companion cells locate near the micropyle. Two others unite to form a large nucleus near the center, while the remaining three, the antipodal cells, pass to the end opposite the egg cell.

Following fertilization, the egg cell begins the initial divisions of the growth of the embryo, but its development is less rapid than that of the large central nucleus, which is the source of the endosperm. The earliest and most vigorous cell division of the endosperm-forming tissue occurs near the distal end of the grain upon the ventral side and adjacent to the antipodal cells which, according to Johannsen,[1] have by this time increased to the number of 30 or thereabouts. After the first layer of endosperm cells has been formed, the growth continues radially toward the center of the grain. The mature stage is always found on the ventral side and particularly in the flanks of the furrow. It is only after the embryo sac is entirely filled with cells that the outer layer of the endosperm begins to differentiate. Although at this time starch grains are infrequent throughout the endosperm, its external layer begins to exhibit the proteolytic

[1] Johannsen, W. Om Frøhviden og dens Udvikling hos Byg. Meddelelser, Carlsberg Laboratoriet, Bd. 2, Hefte 3, p. 103–133, 3 pl., 1884. (French ed., p. 60–77.)

character of the aleurone sheath. The first starch grains are formed in the older areas of the endosperm near the distal end of the grain. According to Brenchley,[1] this occurs on the sixth day after flowering and in the middle of the flanks of the grain. On the seventh day, scattering grains are apparent in two-thirds of the length of the grain. On the tenth day, the deposit is very heavy in the flanks and extends almost to the embryo. It is not until the nineteenth day that the main body of the starch is completed. The cells next to the aleurone layer are the last of the endosperm tissue to fill with starch.

Accompanying this infiltration of starch are certain modifications of the nuclei of the endosperm. Just before the first starch grains are formed the nuclei become granular. For a time they are full of vigor, with the starch grains arranged about them. As the process advances, the nuclei gradually become distorted by pressure exerted by the growing starch. The cells next the aleurone layer and near the furrow are affected much later and to a lesser degree. As above stated, the aleurone layer begins to differentiate soon after the embryo sac becomes filled with new cells. It consists at first of a single layer, increased to three as the endosperm mass grows. The cells are arranged radially and present about the same appearance in both cross and longitudinal section. In tangential section their arrangement is very irregular. Their contents quickly show the characteristic aleurone nature. The dense mass of glutinous and fatty compounds, the aleurone grains themselves, and the large nuclei are present in no other section of the endosperm. As the cells approach maturity the walls become very thick and are seen to be perforated with small canals.

It is only after the cells of the endosperm have divided many times and the endosperm mass is beginning to take shape that the embryo begins active development. At first it lies free in the sap at the lower end of the embryo sac. Gradually the endosperm becomes organized about it, and by the time the more prominent divisions of the embryo have become differentiated the two are in contact. The later growth of the embryo is at the expense of the starch endosperm and during germination their relation is that of parasite to host; but before the grain is matured this statement is not entirely justified, in that, although numerous endosperm cells are emptied of their contents and a mass of collapsed cell walls is crowded before the upper angle of the scutellum, their reduction takes place more in the manner of normal growth. The cell walls are not broken down, and the removal of the starch is effected by the processes typical of the diastase of translocation rather than by that of the corrosive action of digestion.

[1] Brenchley, W. E. Development of the grain of barley. Annals of Botany, v. 26, no. 103, p. 903–928, 22 fig., 1912.

Within the embryo various tissues arise. A primary radicle and from five to eight secondary rootlets, inclosed in their sheathing tissues, are directed toward the proximal end of the grain. The plumule within the plumule sheath points up over the endosperm toward the distal end of the seed. Attached midway between the root and plumule—that is, to the hypocotyl—by a wide ligament, or umbilicus, is the scutellum. This organ spreads out over the entire surface of the endosperm lying next to the embryo and has on its inner or adjacent surface a layer of specialized cells—the epithelial layer.

GERMINATION.

Germination is the continuation of the growth of the embryo, arrested at the time of the maturation of the seed. The first step in germination is a distension of the grain, due to the absorption of water. In the resting stage most of the tissues of the embryo are free from starch. When first brought under conditions favorable for germination the few starch grains present disappear. Very shortly, however, starch again becomes evident in tissues that were perfectly free from it at maturity. In the outer layers of the scutellum a marked deposit is soon apparent. As the process advances, the cells of the epithelial layer elongate slightly. Brown and Morris [1] noticed that one of the first changes in the grain was in the appearance of the protoplasm of these cells. In the resting state this is clear and finely granular. As the first steps of germination are in progress it assumes a much coarser character and the cell becomes cloudy. This condition obtains until the endosperm is almost entirely absorbed.

The first actual formation of new tissue takes place in the primary radicle. This breaks through the coleorhiza and is the first part of the plant to emerge from the grain. The secondary rootlets are slightly less advanced. The plumule develops more slowly, and under restrained malting conditions it may be several days in reaching less than the length of the grain.

In the endosperm, germination is, of course, a process of destruction. In the history of a single cell the walls first become thickened as if distended with water. The laminæ become distinct, the walls become translucent, and disintegration sets in. Various investigators have asserted that a complete obliteration of the walls takes place, but the writers have not been able to arrive at this conclusion, the walls always being discoverable to some extent, especially by means of staining. As soon as they have been sufficiently weakened, action begins upon the starch grains. Their dissolution in this case is not by the translocation process, in which they gradually become

[1] Brown, H. T., and Morris, G. H. Researches on the germination of some of the Gramineæ, part 1, Journal, Chemical Society [London], v. 57 (Transactions), p. 458–528, 1890.

smaller while still retaining their uniform shape, but by the corrosive action of a powerful digestive diastase. The grains become irregularly pitted and are rapidly absorbed.

The cells first affected are those in contact with the scutellum. According to Brown and Morris the first visible change in the appearance of the starch grains in the endosperm cells is coincident with the first appearance of starch in the cells of the scutellum, immediately back of the epithelial layer. After the first layer of endosperm cells has been broken through, the process gradually extends through the remainder of the endosperm. The action takes place along three lines: (1) It slowly proceeds directly into the mass of the starch endosperm; (2) it moves with more rapidity through the area immediately adjacent to the aleurone layer; and (3) it follows along the furrow at an even greater rate. The course of attack is thus directly opposite to that of the infiltration of starch. The cells last to receive deposits of starch are the regions of most rapid depletion. At the end of four or five days' slow germination, the path of affected tissue appears in longitudinal section as more or less of a crescent, the horns well advanced beneath the aleurone layer. Indeed, the crescent is already apparent in Plate II, figure 2, where germination was arrested at the end of the second day.

As germination advances, the tissues earlier affected become almost entirely liquefied and the diastatic and cytatic action proceeds to the reduction of the remaining endosperm areas. The last parts to yield are the dense deposits in the center of the flanks of the grain and especially those farthest from the scutellum. The epithelial layer undergoes some significant modification during this progressive starch solution. As previously noted, the cells first elongate slightly and certain changes take place in the protoplasm. Later there occurs (and more or less rhythmically reoccurs) a deposit which gathers in the outer ends of these cells. When the digestion is almost complete, the epithelial cells undergo a sudden elongation, attaining a length almost four times that which marked their former condition.

In the process of malting, the aleurone layer is not affected. It is found intact in brewers' grains even after they have endured the heating and strong diastatic action of the mash tub. In normal growth it is only when the plant has developed to the point of exhausting the starch endosperm that this layer begins to disintegrate and is absorbed by the plant.

CONVERSION OF THE ENDOSPERM.

The morphological changes in the endosperm are, of course, only the visible effects of the essential actions, which are enzymatic. The disintegration of the cell walls is a chemical transformation of cellulose to sugar and other compounds. The breaking down of the

starch is the result of the action of maltose-forming diastase. The disappearance of the plasmic network of the cells is due to its being peptonized by proteolytic enzyms and thus made soluble for absorption. Chemistry has made known the processes by which these changes are effected. It has found methods to increase the power of the grain to digest these various substances. It has invented ways of manipulation so as to secure the greatest efficiency of the enzyms present. It has not, however, shown any way of increasing the capacity of a grain to secrete enzyms, nor has it discovered any modification of structure that would lead to a greater economy in conversion.

It is only by a definite location of the sources of these energies that a working basis for improvement of the barley grain can be obtained. Not only must it be known which of the tissues are concerned in these activities, but the extent and nature of the action of each tissue must be understood. Upon this point there has been much divergence of opinion. The disagreement has been not only as to the parts of the grain concerned, but also as to whether or not the various enzyms are secreted by the same or by separate organs. Since there are three areas in dispute and three enzyms to be accounted for, the number of possible hypotheses has occasioned a very considerable confusion.

RÉSUMÉ OF THE CONCLUSIONS OF OTHER INVESTIGATORS.

Although the number of investigators who have been interested in one or several phases of this subject is great, a brief statement of their conclusions is necessary at this point.

Krabbe [1] assigns all diastase secretion to the individual cells of the endosperm, based on his assertion that diastase can not pass from cell to cell.

Green [2] finds in *Ricinus communis* a marked ability of the endosperm for self-digestion.

Hansteen [3] finds that the endosperms of *Zea mays* and *Hordeum vulgare* are capable of self-digestion when products of conversion are removed, that the aleurone does not supply the diastase, and that the scutellum is capable of vigorous corrosion of adjacent starch.

Pond,[4] in an exhaustive chemical research, fails to discover any ability for self-digestion in the date.

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[1] Krabbe, G. Untersuchungen über das Diastaseferment unter specieller Berücksichtigung seiner Wirkung auf Stärkekörner innerhalb Pflanze. Jahrbücher für Wissenschaftliche Botanik [Pringsheim], Bd. 21, Heft 4, p. 521–561, pl. 13–15, 1890.

[2] Green, J. R. On the germination of the seed of the castor-oil plant (Ricinus communis). (Abstract.) Proceedings, Royal Society, London, v. 47, no. 287, p. 146–147, 1890.

[3] Hansteen, Barthold. Ueber die Ursachen der Entleerung der Reservestoffe aus Samen. Flora, Bd. 79, p. 419–429, 1894.

[4] Pond, R. H. The incapacity of the date endosperm for self-digestion. Annals of Botany, v. 20, no. 77, p. 61–77, 1906.

Puriewitsch[1] concedes no more power to the aleurone layer than to the rest of the endosperm.

Torrey,[2] in studies of germinating maize, attributes the secretion of both cytase and diastase to the epithelial layer. He associates the enzym with the production of a definite granular substance in the epithelial cells the formation of which is coincident with the first attack upon the endosperm cells. Incidentally, he attributes the depleted layer of crushed cells, which is formed in the later stages of growth, to pressure and not to the absorption of the still growing endosperm by the expanding scutellum.

Van Tieghem[3] finds, among many things less pertinent to this discussion, that the embryo of *Mirabilis jalapa* is capable of utilizing macerated endosperm as a nutritive substance.

Sachs[4] considers, except for minor details, that the relation of embryo to endosperm is one of parasite to host.

Linz[5] finds that the removal of the aleurone layer of corn makes little difference in the dissolution of the endosperm. He concludes that this layer is not secretive.

Grüss[6] finds that the scutellum of barley is able to secrete abundant diastase and to nourish itself upon starch supplied in place of the endosperm.

Reed[7] looks upon the epithelial layer as containing the only secreting cells in either *Zea mays* or *Phoenix dactylifera*, but concerns himself mostly with the changes in nuclear and plasmic conditions.

Brown and Escombe[8] conclude that the scutellum of barley secretes diastase, but that the production of cytase occurs in the aleurone layer, but they do not find any power of secretion in a detached aleurone layer.

Green[9] assigns the origin of cytase for the most part to the aleurone layer, but attributes the production of the greater part of the diastase to the scutellum.

Brown and Morris,[10] in one of the most exhaustive investigations to which these questions have been subjected, find the scutellum of

[1] Puriewitsch, K. Physiologische Untersuchungen über die Entleerung der Reservestoffbehälter. Jahrbücher für Wissenschaftliche Botanik [Pringsheim], Bd. 31, Heft 1, p. 1–76, 1897.

[2] Torrey, J. C. Cytological changes accompanying the secretion of diastase. Bulletin, Torrey Botanical Club, v. 29, no. 7, p. 421–435, pl. 20, 1902.

[3] Van Tieghem, P. E. L. Recherches physiologiques sur la germination. Annales des Sciences Naturelles, Botanique, s. 5, t. 17, p. 205–224, 1873.

[4] Sachs, Julius von. Lectures on the Physiology of Plants. Tr. by H. Marshall Ward. Oxford, 1887, p. 373.

[5] Linz, F. Beiträge zur Physiologie der Keimung von Zea mais. Jahrbücher für Wissenschaftliche Botanik [Pringsheim], Bd. 29, Heft 2, p. 318, 1896.

[6] Grüss, J. Ueber die Secretion des Schildchens. Jahrbücher für Wissenschaftliche Botanik [Pringsheim], Bd. 30, Heft 4, p. 645–664, 1 fig., 1897.

[7] Reed, H. S. Study of the enzym-secreting cells in the seedlings of Zea mais and Phoenix dactylifera. Annals of Botany, v. 18, no. 70, p. 267–287, pl. 20, 1904.

[8] Brown, H. T., and Escombe, F. On the depletion of the endosperm of Hordeum vulgare during germination. Proceedings, Royal Society, London, v. 63, no. 389, p. 3–25, pl. 1, 1898.

[9] Green, J. R. Soluble Ferments and Fermentation. Cambridge [England], 1899, p. 29, 93.

[10] Brown, H. T., and Morris, G. H. Researches on the germination of some of the Gramineæ, part 1. Journal, Chemical Society [London], v. 57 (Transactions), p. 458–528, 1890.

barley capable of converting certain starches supplied in the place of its endosperm. They find that excised embryos are able to grow when the scutellum surface is grafted on endosperms in which life is extinct. They consider the endosperm no longer a vital tissue and claim that the aleurone layer does not secrete enzyms, assigning cytase, as well as diastase, to the scutellum.

Ling [1] supports Grüss, as opposed to Brown and Morris, regarding the extent of the dissolution of cell walls in conversion. The latter attribute the "mealiness" of malt to a marked dissolution of the cell walls. Grüss holds that the walls become merely transparent. Ling finds the walls present in all parts of the endosperm, although affected by cytase.

Vines,[2] while working with yeast and agarics, assumes that similar enzyms exist in other plants. He finds two classes of enzyms: (1) Those that peptonize and (2) those that peptolyse.

Beaven,[3] while only incidental to the subject of his paper, remarks that the starch endosperm of barley is built up in centrifugal order and that its outer layer is easily broken down.

Gibbs [4] states that in the Alsinoideæ the greatly reduced endosperm is active only as an absorptive organ of the food material which is here stored in the perisperm.

Ellrodt [5] finds that small-berried malts give a higher diastatic power per gram of original material than do large-berried ones.

Brown [6] finds that the semipermeable membrane exercises a selective action, allowing water to enter the grain, but absolutely excluding most substances in aqueous solutions. The absorption is uniform in all parts of the grain.

SOURCE OF DIASTATIC FERMENTS.

On account of the fact that starch is the form in which most of the convertible material is stored, its digestion has received a great amount of attention. The process has been ascribed to three sources: (1) To a secretion of the endosperm itself; (2) to a secretion by the aleurone layer; and (3) to a secretion by the scutellum.

The theory of the self-digestion of the starch endosperm has been conspicuously championed by only two men, namely, Hansteen [7]

[1] Ling, A. Presence of cell walls in the endosperm of malts. (Abstract.) Brewers' Journal [New York], v. 29, no. 10, p. 440, 1905.

[2] Vines, S. H. Proteases of plants. Annals of Botany, v. 18, no. 70, p. 289–317, 1904.

[3] Beaven, E. S. Varieties of barley. Journal, Federated Institute of Brewing, v. 8, no. 5, p. 542–600, 12 fig., 1902.

[4] Gibbs, L. S. Notes on the development and structure of the seeds in Alsinoideæ. Annals of Botany, v. 21, no. 81, p. 25–55, 4 fig., pl. 5–6, 1907. Abstract in Botanical Gazette, v. 43, no. 5, p. 349–350, 1907.

[5] Ellrodt, G. Unterschied des Diastasegehaltes von Malzen aus grosskörnigen und kleinkörnigen Gersten. Wochenschrift für Brauerei, Jahrg. 23, no. 20, p. 243–244, 1906; also in Zeitschrift für Spiritusindustrie, Jahrg. 29, no. 23, p. 209–210. Abstract in American Brewers' Review, v. 20, no. 7, p. 379, 1906.

[6] Brown, A. J. On the existence of a semipermeable membrane inclosing the seeds of some of the Gramineæ. Annals of Botany, v. 21, no. 81, p. 79–87, 1907.

[7] Hansteen, Barthold. Ueber die Ursachen der Entleerung der Reservestoffe aus Samen. Flora, Bd. 79, p. 419–429, 1894.

and, later, Pfeffer [1] in his review of Hansteen's work. The work of Hansteen is of some significance. He found that when the products of conversion were constantly removed, the endosperm was able to digest itself. His conclusion, however, that the endosperm is entirely capable of self-digestion is rather too sweeping, especially since he himself found a very active secretion of diastase in the scutellum, the existence of which function is hard to understand if it be superfluous. It is much more probable that the method used by Hansteen merely exaggerated the normal action of such diastase as would naturally be expected to be present in the endosperm of the ripened grain, for a certain minute amount of diastase is necessarily present in the cells of the endosperm to carry on the process of starch infiltration. This is, however, merely that phase of diastase needed for the translocation of the starch while the barley grain is forming. Indeed, according to the figures of Brenchley, the amount of diastase in the growing grain decreases as the grain approaches maturity. It is even reasonable to expect that not only would traces of this enzym remain potentially functional in the endosperm of the ripened seed, but that the cells of this endosperm, once able to produce this ferment, would retain such ability to some degree, even in their less active condition. The effect of such small quantities of local diastase is not, however, to be confused with the vigorous attack that actually takes place in the process of germination. The conclusions of the writers are supported by those of many investigators, as above cited.

The theory that the aleurone layer is active in endosperm reduction has more in its favor than has that of the self-digestion of the endosperm. The aleurone layer is obviously high in vital energy. Its cells have the unmistakable aspect of active protoplasm. Their nuclei are large and present a sharp contrast to the distorted plasmic centers of the endosperm cells. The aspect of this layer during germination also lends much to the support of the hypothesis of its being functional. As previously noted, it is not digested with the endosperm, but persists until the endosperm is almost entirely absorbed. When germination is carried only to that point where the purposes of malting have been attained, the aleurone layer is still intact. As shown in figure 3, almost perfect sections of this tissue may be obtained from ordinary dried brewers' grains, in which the entire starch endosperm has been destroyed. In still further support of the idea, the disintegration in a newly germinating seed proceeds most rapidly directly adjacent to the aleurone layer. At certain stages this diastatic movement may be seen to have progressed until the thickest part of the endosperm is in a way surrounded with digested starch cells.

[1] Pfeffer, Wilhelm. Untersuchungen von Herrn Barthold Hansteen im botanischen Institut ausgeführten, über die Ursachen der Entleerung der Reservestoffe aus Samen. Berichte, Koniglich Sachische Gesellschaft der Wissenschaften, Leipzig. Mathematisch-physische Classe, Bd. 45, p. 421–428. 1893.

Tangl [1] has shown that the thickened cell walls in the endosperm of many seeds are provided with openings, so that the cell contents are not isolated but in intimate contact. This is true of the aleurone layer of barley, and the layer is in consequence more nearly a unit than might be supposed.

The accumulative importance of these and other facts has caused many authors to assert that the aleurone cells have a very important secretive function. The views of the individual experimenters who are inclined to this opinion are too numerous to discuss separately. The points of variance between them and the writers are few and turn largely on the interpretation of specific phenomena.

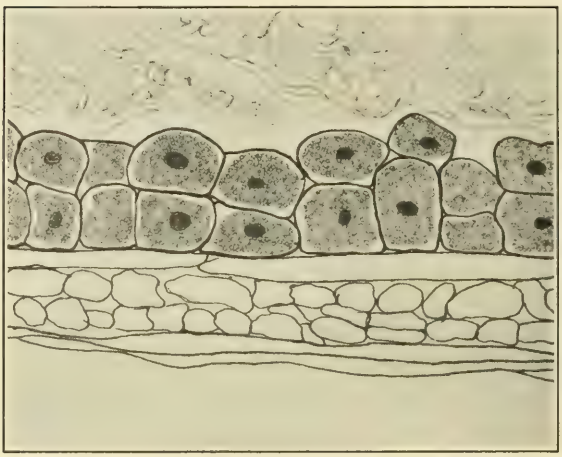


FIG. 3.—Section of piece of "brewers' grain," that is, of a barley grain that has passed through both the malting and the mashing processes. The aleurone layer remains unchanged. The cell contents are slightly shrunken by heating in the mash tub. The starch endosperm is fully depleted of its contents.

These differences really center around a single fact. When the breaking down of the starch endosperm once commences next to the scutellum, it follows rapidly through the cells immediately beneath the aleurone layer. This point is frankly admitted, but is far from being a proof of aleurone activity. It has other interpretations more consistent with the general facts of germination. These cells adjacent to the aleurone layer are markedly different from those of the rest of the endosperm. They are younger. The starch endosperm is laid down and its cells filled in centrifugal order, so that its outer areas are the latest in formation and the latest in starch infiltration. They are also less gorged with starch. A grain of barley is not definitely limited in growth. In a way its growth is indeterminate, its development progressive until stopped by the act of ripening, a stage in which the failure of the supply of food is a marked factor. As may be seen in Plate III, the outer cells must from their very nature be younger and less filled with starch, their walls less desiccated, and their nuclei in a more nearly normal condition than those of the more thoroughly matured storage cells. A cross section

[1] Tangl, Eduard. Ueber offene Communicationen zwischen den Zellen des Endosperms einiger Samen. Jahrbücher für Wissenschaftliche Botanik [Pringsheim], Bd. 12, Heft 2, p. 170–189, pl. 4–6 [1880].

of a ripe barley grain treated with Millon's solution presents a strongly colored band just inside the aleurone layer and completely surrounding the rest of the endosperm, showing the relatively high proteid and low carbohydrate content of this region. The cell contents, therefore, are obviously not identical with those beneath.

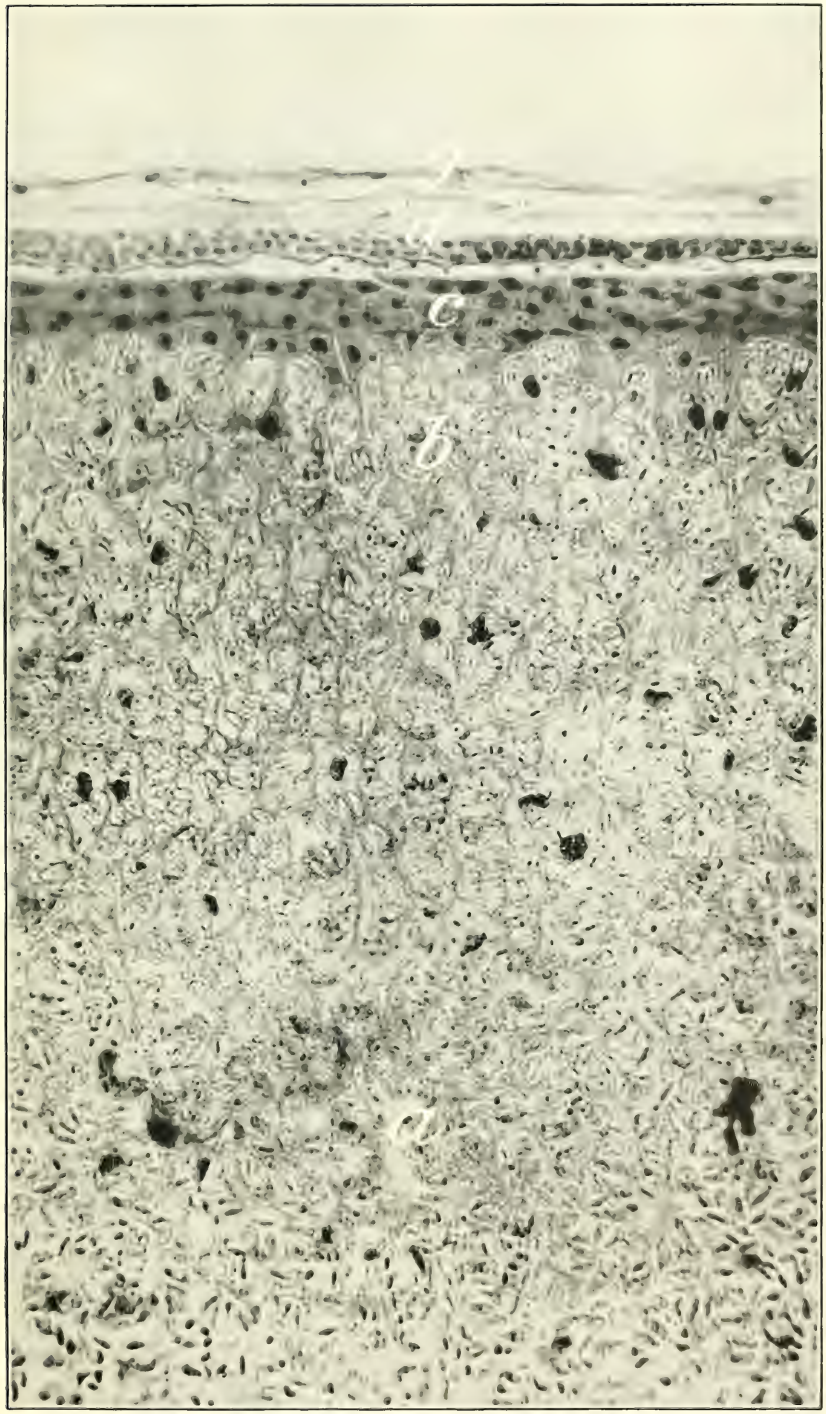
It is but reasonable to suppose that these young and loosely filled cells would be more easily broken down and that diastase, acting from the proximal end of the grain, even if distributed equally over the surface of the endosperm, would advance most rapidly along this area of least resistance. A strong point against the aleurone theory of digestion is the fact that the cells of the endosperm first affected are those next to the scutellum, as shown in Plate IV. If the aleurone layer is active, why should not this take place uniformly in those cells lying directly beneath that layer? Sections made through grains germinated at high temperatures show the proximal end of the endosperm to be in complete solution, while the cells next to the aleurone layer toward the distal end of the grain are entirely unaffected. This can not be due to a lack of water, for water can be shown to pass readily through the walls of all parts of the grain. One or two authors have attempted to ascribe this condition to a certain inexplicable stimulus which proceeds from the growing plantlet and passes through the connected plasma of the aleurone cells, inciting them to successive action. Such theory fails to explain why the movement advances much more rapidly along the furrow than elsewhere. The cells in this region are for the most part dead tissue and would be incapable of aiding the aleurone layer or of carrying any stimulus. However, since the region of the furrow contains the conducting tissue during the development of the grain and since it leads past the scutellum, it is readily seen that it could be still instrumental in carrying secretions from that source, although incapable of conveying stimuli.

A more plausible defense of the theory of aleurone secretion has been advanced in the statement that the enzyms are able to work only when the products of their conversion are removed, and that the scutellum as an absorbing organ is responsible for the fact that the action commences adjacent to it and can not proceed elsewhere, except as the process of removal becomes effective. This theory is not borne out by observations, in that the lack of a means of removal would not prevent the preliminary stages of dissolution from taking place at all points in contact with the aleurone layer. No such effect occurs. Moreover, the percentage of maltose necessary to the inhibition of diastatic action is very high, certainly not less than 30 per cent. That this percentage is seldom reached is shown by the fact that the cells first acted upon while in a partial state of conversion pass on to the point of complete dissolution, even though they

are gorged not only with their own products of conversion but with those of the cells lying beyond them.

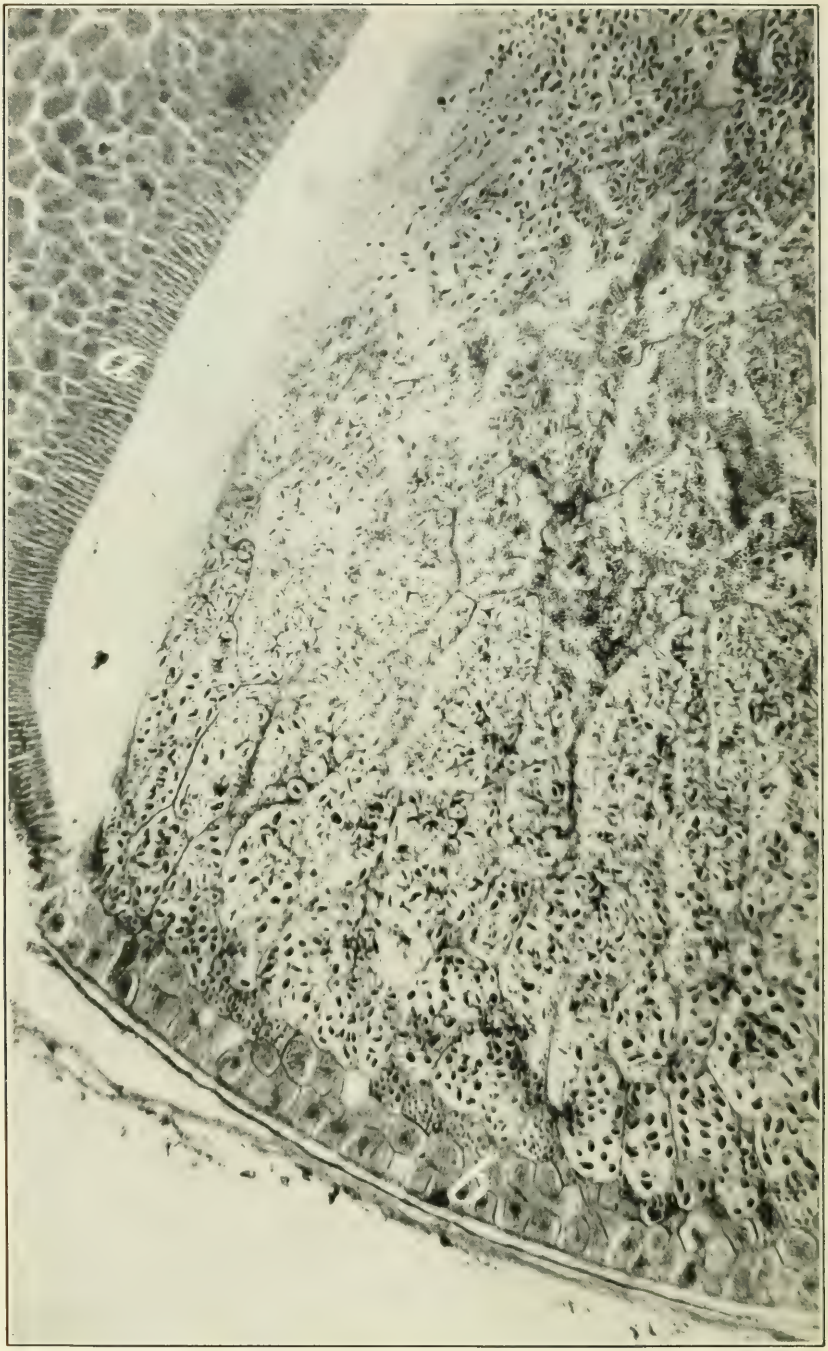
As must be evident from the writers' opposition to the theories of self-digestion and of aleurone activity, their investigations have been overwhelmingly in favor of placing the entire function of diastase secretion during germination upon the scutellum. Morphological examinations have been made of thousands of barley grains at all stages of germination. These have been repeated with a large number of varieties and types of barleys grown under varying conditions in America and of seed imported from all parts of the world. In all cases, the dissolution of the endosperm is effected in the same manner. The corrosion commences with the parts directly in contact with the scutellum. The action begins as quickly in front of the cells in the center of the scutellum as it does in those next to its periphery and therefore in juxtaposition to the aleurone layer. All later action is such as would occur if the scutellum were the only source of diastase. A grain from which the aleurone layer is removed is converted in the usual manner. Moreover, the very appearance of the scutellum is convincing. On its surface is the epithelial layer, which is typically glandular. There are in plant growth no tissues that closely resemble specialized secreting tissues, and seldom is there a more unmistakable development of this than the epithelial layer, as shown in Plate V, figure 1.

Most investigators have recognized the secreting power of the scutellum; indeed, none have denied such function, even when attributing to it only a subsidiary activity. Numerous experiments have been made in the past and many of them repeated by the writers. A grain from which the embryo is removed will not show any tendency to germination, even though all external conditions, such as light, heat, and moisture be made favorable and ample provision for the removal of conversion products be made. On the other hand, and under proper artificial conditions, the scutellum is able to support itself and to supply food for the growth of the plumule and radicle independent of the endosperm. Morris and Brown, Hansteen, Pfeffer, and numerous others have shown the ability of the embryo (with the scutellum) to digest the starch supplied to it. This has been demonstrated in numerous ways. The starch has been furnished in the form of macerated endosperm and in the form of a mixture of starch and plaster. In every case, the scutellum was able to corrode the material supplied and to promote a considerable growth in the plantlet. The embryo is also able to utilize an endosperm which is entirely without living protoplasm. An excised embryo from a healthy, vital grain can be grafted upon a dead endosperm with perfect success. Such endosperm may be prepared in any way desired, even by prolonged soaking in absolute alcohol; that is to say, after



PHOTOMICROGRAPH OF A LONGITUDINAL SECTION OF A PORTION OF A BARLEY GRAIN IN THE EMBRYONIC STATE.

a, Inner area of the endosperm, with starch grains; *b*, outer and younger area just forming, with few starch grains and large nuclei; *c*, aleurone layer; *d*, integuments and pericarp; *g*, fragments of glumes.



PHOTOMICROGRAPH OF A PART OF THE SCUTELLUM AND ENDOSPERM OF A GERMINATING BARLEY GRAIN.

The initial stage of germination is shown. Enzymatic action has begun in front of the epithelial layer (*a*), but not next the aleurone (*b*).

a submersion for several months in this killing fluid a living embryo is able to effect the usual processes of conversion.

That the functions of the scutellum are localized in the epithelial layer is readily shown by experiment. The removal of this layer immediately results in an absolute loss of the scutellum's power of digestion. It is so fully incapacitated that no visible corrosion is effected upon a contact surface of starch, when previously such effect was very marked. An epithelial layer, even when not connected with the embryo, seems able to accomplish a slight change, even though no provision for the removal of the products of conversion is made.

The exact way in which the epithelial layer produces diastase is not known. The change in the plasma, the elongation of the cells, and the peculiar granular deposit that accumulates at their outer ends are all probably connected with the exercise of this function. Indeed, Torrey has gone so far as to assert that this is a deposit of actual enzymatic substance. He asserts that it appears, gathers at the outer end of the cell, and then passes into the endosperm, that the cell stays clear for some time, and that the same phenomena are then repeated. The writers have not verified this statement beyond noting that the deposit varied greatly in individual grains which were killed at different stages of germination. Sometimes the entire end of the cell was clouded and the outer end heavily charged with a coarse granular deposit, and at other times it was entirely free.

LOCATION OF DIASTASE SECRETION.

There are two diastases present in the barley grain: One of translocation, capable of only weak action; and one of digestion, capable of powerful corrosion.

The endosperm is incapable of self-digestion other than the slight action of the diastaste of translocation.

The aleurone layer is made up of highly vital cells, which persist until the starch endosperm has been almost completely absorbed.

The most rapid starch conversion is next to this layer.

These facts are not sufficient to ascribe any secretive function to the aleurone layer, the rapid dissolution next to this tissue being due to the fact that from their nature the adjacent cells are easier to break down than the ones in the central part of the endosperm. An even more rapid corrosion follows the furrow, although it is largely composed of inactive and dead cells. No corrosion is to be found next to the aleurone layer except as the changes move outward from the scutellum. The absence of means to remove the products of conversion at the distal end should not prevent the first stage of conversion taking place if the aleurone layer were an active enzymatic organ.

That the scutellum is active in enzym secretion is shown by the fact that the first changes occur in the layer of endosperm in contact

with it, and all later progress in conversion is made from it toward the distal end of the grain.

The embryo is able to feed upon starch substituted for its normal endosperm. Living embryos are able to grow when grafted on endosperms which have been previously killed.

The function of enzym secretion in germination is localized in the epithelial layer of the scutellum. Embryos from which this layer has been removed are unable to convert starch.

SOURCE OF CYTATIC AND PROTEOLYTIC FERMENTS.

The location of the secretion of diastase is not the only point in the physiology of the grain upon which investigators have differed. Some authorities who have placed this function in the scutellum have ascribed the formation of cytase and of the proteolytic enzym to other sources.

With cytase, if the conclusions of the writers be correct, the question is soon settled. If starch conversion starts next to the embryo and moves toward the distal end of the grain, cytase must also be a product of the epithelial layer. The breaking down of the cell walls must always precede the action of diastase. The diastase of digestion seems to be unable to pass readily through unmodified cell walls, and most certainly the cell walls are not affected much beyond the depleted zone. These two enzyms, then, must of necessity not only be associated in the changes which occur in the endosperm, but must proceed from a common source.

The proteolytic ferments would appear from analogy to present no differences from the other two. Proteolytic action certainly does not occur in any part of the endosperm not yet reached by cytase and diastase. If the proteolytic ferments are secreted by the aleurone layer they do not become operative until the cytase has moved forward from the scutellum and has broken down the cell walls. This lack of plausibility is not proof, but, on the other hand, there is no reason for thinking that there is more than the single source of enzymatic secretion. In one or two other grasses any other origin than the scutellum is apparently impossible, because the aleurone layer is digested almost as readily as the starch endosperm. In these cases, at least, the secretion must be a product of the scutellum, and in barley every indication short of absolute proof insists on the same conclusion. In the opinion of the writers, the scutellum as a feeding organ is endowed with all the functions of digestion, being able to utilize all foods occurring in its natural storehouse, the endosperm.

FUNCTION OF THE ALEURONE LAYER.

These conclusions leave unexplained one very evident fact. The aleurone layer is obviously a vital tissue. Its cells are in no way similar to those of the starch endosperm beneath it. This marked

difference can not be without a purpose, and fortunately this purpose is easily explained without the necessity of having recourse to any theory of secretion. The aleurone layer undoubtedly exercises a strong protective function. In rainy weather, fungi are invariably to be found investing the barley grain and feeding upon the feebly active cells of its envelopes. Their hyphæ often run through all the investing layers of the seed without being able to penetrate the aleurone layer. Whether or not it be the vital resistance of its protoplasm, the mere mechanical obstacle of its heavily cuticularized walls, or both, this tissue is an effective barrier to the inroads of molds and bacteria. The fact that such protection is of absolute necessity is readily appreciated. Within the endosperm is stored the food upon which the future development of the embryo is absolutely dependent. Even the perpetuation of the species rests upon the proper conservation of this reserve food material. This mass of readily assimilated compounds is an ideal food for all sorts of saprophytic and parasitic organisms. It is therefore not surprising to find built around this material a specialized tissue with a highly protective function.

The aleurone layer serves a second purpose, but one which alone would hardly justify its existence, in that it appears to be more opportune than essential. When the plant is first establishing itself in the soil, it utilizes the starch endosperm, a material rich in carbohydrates, which seems to meet all the requirements of early growth. When once its green leaves are exposed, however, photosynthesis is able to supply all its needs for starch. With nitrogenous food material the problem is more difficult. A considerable extension of feeding surface in the roots is necessary before such material can be procured in any quantity. The starch endosperm contains an extremely limited amount of nitrogenous matter and that stored in the embryonic tissues is soon exhausted. The breaking down of the aleurone layer and the utilization of its highly nitrogenous cell contents comes at the critical period in the life of the plantlet when this material is of especial value. This may account for the extra thickness of this layer in certain genera of grasses, of which Hordeum is one of the most notable examples.

GREATER DIASTATIC POWER OF SMALL-BERRIED AND OF HIGH-NITROGEN BARLEYS.

It is interesting to note that the theory of scutellar secretion conforms nicely to an apparently satisfying explanation of a widely recognized fact in the utilization of barley malt. Distillers have long known that their requirements are best met by a malt of high diastatic power and that small-berried barleys of high nitrogen content are best adapted to produce such malt. With those brewers who are producing a beer in the manufacture of which malt adjuncts, such

as corn grits or brewers' rice, are used in conjunction with malt, the same type of malt is popular, although an extreme development of diastase is not here demanded, because the additional amount of starch to be converted is less.

The explanation of the greater enzymatic power of small-berried barleys seems simple. The diastase-secreting surface is confined to the epithelial layer. The proportion of epithelial surface to volume of endosperm should also represent the ratio of diastase to starch. For the purpose of illustration, the shape of the barley grain may be considered as a sphere, while the curve of the scutellum may be taken to represent that of a second sphere partly included in the first. If the size of the grain is made greater, its volume is increased much more rapidly than the area of the surfaces of the spheres, according to well-known laws of geometry. That is to say, for any increase in the size of the grain the increase in the area of the surface of the scutellum is much less proportionately than the increase in the volume of either the embryo or the endosperm; and, conversely, as a grain decreases in size the epithelial surface decreases much less proportionately than the bulk of its endosperm. It is therefore but natural that the diastase production in a small grain should be relatively greater than in a large one and often greatly in excess of the needs for conversion.

The relation of diastatic power to nitrogen content is a slightly more complex problem. In general, barleys high in nitrogen are also high in enzymatic power. The case varies somewhat with conditions. In the small-berried barley the same facts apply as in the ratio of scutellar surface to endosperm. The aleurone layer is one of the great sources of nitrogen in the barley grain. This layer is almost invariably three cells deep. If the grain be very small, the two or three layers of cells completely encircling the starch endosperm form a very considerable part of the grain. Since they are high in nitrogen, this element tends to represent a higher and higher percentage of the total as the diameter of the grain is progressively reduced. In a very small grain the percentage of nitrogen is therefore likely to be high, even though the starch endosperm be ever so mealy, the deciding factor being, of course, the great proportion of the aleurone layer.

There is, however, among the 2-rowed barleys a different situation, in that climate and culture assume a greater importance. Barley-growing areas are often conspicuous for their production of high-nitrogen barleys. Indeed, the crops of any section vary in this respect from year to year. It is almost invariably the case that these high-nitrogen crops are also high in diastatic power, often possessing an enzymatic potency almost equal to that of the small-berried sorts. In these cases, also, the explanation is found in the ratio of the

secreting surface to the starch endosperm. The growth of the grain in such cases is not normal. The stimulation of the vegetative growth has been greater than the later starch infiltration could balance. The embryo and general structure of the grain have attained large proportions, but the starch infiltration has been incomplete. A normally grown and matured grain of 2-rowed barley possesses a highly developed starch endosperm. Although this endosperm is the first part of the grain to commence active growth, it is the last to be completed in the process of ripening. If any feature of climate or culture interferes with the process of starch deposition, the grain remains high in nitrogen content. This excess is due more to the absence of starch than to the presence of nitrogen. There is, then, a large scutellum without the corresponding bulk of starch. The enzymatic secretions intended for the conversion of a large endosperm are more than sufficient for the reduced starch content, and both diastatic power and nitrogen content are higher than usual.

In substantiation of this view, measurements were made upon two samples of Princess barley. These were grown at Huntley, Mont., in 1911. One sample was produced upon dry land, while the second came from a neighboring plat which had been irrigated. The thousand-berry weight of the irrigated barley was 45.5 grams, while that from the dry-land plat was only 30.1 grams. The scutellums in either case were quite uniform. Those from the irrigated sample averaged a little less than 2.6 millimeters in diameter, while those from the dry-land sample fell to 2.4 millimeters. The scutellar areas were, then, 5 square millimeters for the one and 4.3 square millimeters for the other. That is to say, under such conditions that the grain did not develop to its fullest extent the scutellar area was reduced approximately 14 per cent. However, at the same time, the total reduction of the grain was 33 per cent. As the hulls are more or less a constant factor, the actual reduction of endosperm was probably far in excess of 33 per cent. This case is but a slight exaggeration of the conditions of incomplete development that usually obtain over a considerable part of the upper Mississippi Valley. The scutellum of 2-rowed barleys in this section is, for the most part, likely to be relatively a greater part of the grain than is normally the case in this group. Such barleys grown in this section are likely to show a much greater converting power than has been suspected.

EFFICIENCY OF CONVERSION.

If the conclusions of the writers be correct, the application of these facts to barley production and utilization is of much importance. If the secretion of enzyms takes place in the epithelial layer, the gross morphology of the grain must govern its behavior in germination, including that modified form of germination known as

malting. The perfection with which an endosperm undergoes digestion depends upon the amount of diastase present and upon the location of the endosperm with reference to the diastatic source. It is the aim of malting to carry the disintegration of the endosperm only to that point where cytatic action has been completed and where diastase formation has reached a large total which has been distributed through the grain. Very little starch conversion is desired in the process of malting. Indeed, none is desired, since the sugars thus produced are in part absorbed by the growing plant and not only lost but changed into undesirable products. The low temperatures at which the grain is malted are for the purpose of restraining this conversion. Every effort is made to prevent the absorption by the plantlet of the contents of the proximal region of the endosperm before the enzymatic secretions have reached its distal end and have permeated the dense starch areas in the upper flanks of the grain. When this is attained the embryo is killed to prevent any further absorption. The object of malting is, therefore, to subject all parts of the endosperm to the initial effects of enzyms with as little loss as possible. The ideal condition would be a simultaneous attack upon every cell wall and every grain of starch. This is impossible with the source of the action localized at one extremity of the grain. However, the coordination of the scutellum with the endosperm has a marked effect.

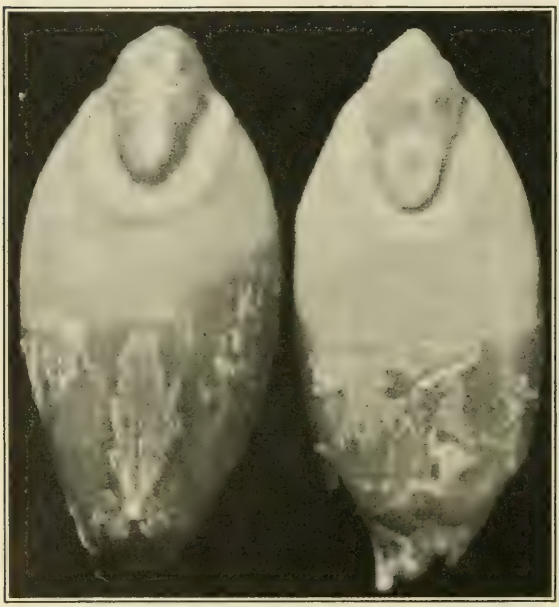


FIG. 4.—Two grains of a Bohemian barley; a broadly oval-shaped grain well adapted to malting with little loss.

The shape of the grain with reference to the size and shape of the associated embryo has in more than one variety been the deciding factor between a good and a poor malting barley. If the great mass of the starch be near the embryo, as in the short, thick grain shown in figure 4, the disintegration of the endosperm is readily and uniformly accomplished, but if it be distant, as in long, slender grains, the complete modification of the endosperm mass can not be accom-

plished without great loss. That is, the more nearly a grain approaches a sphere in shape, the less need be the loss in malting; and the greater the ratio of the longer axis of the grain to the shorter the more difficult becomes the malting. In extreme types, such as the Chinese barley shown in figure 5, efficient malting is impossible. If the process is stopped in a reasonable time, there remains an unaffected portion at the distal extremity, whereas if germination is continued until this portion becomes softened, the entire proximal end will have been utilized by the plant. The 2-rowed barleys from Smyrna are less marked examples of the same defect. They are almost ideal in character, considered from the standpoint of high extract alone, except for their unfortunately long grain. The shape of the grain makes this barley difficult to malt, and the extract is probably measurably less than would be the case if the same seed contents were differently arranged. Barleys with long grains and with pointed ends are to be avoided for malting purposes, if yield of extract be even of secondary interest.

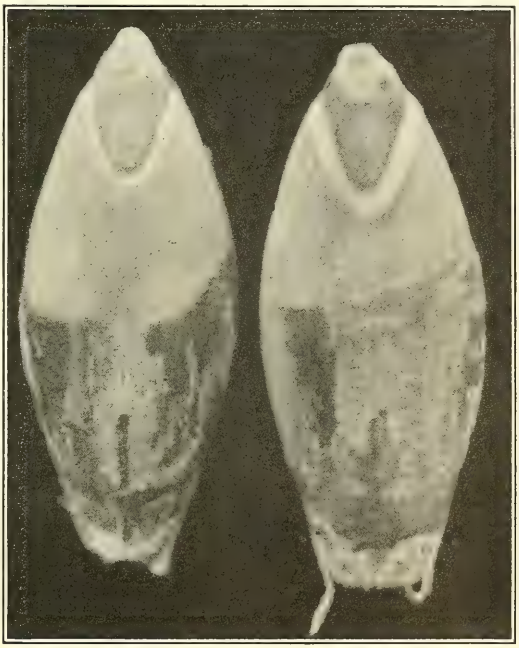


FIG. 5.—Two grains of a Chinese barley, a spindle-shaped grain very difficult to malt.

The amount of diastase present in any type of barley must depend upon the area and vigor of the secreting surface. If the secreting surface be large, enzymatic action should be ample. As previously pointed out, the proportion of the secreting surface must, by the laws of geometry, increase as the size of the berry decreases. Thus, in small-berried barleys the diastatic power will be high, regardless of the nature of its endosperm. If, in addition, the endosperm be dwarfed by a lack of normal starch infiltration, resulting in a high-nitrogen grain even for its class, the excess of diastase becomes still greater and results in a malt adapted to the use of distillers. The question of increasing this function in such barleys thus seems superfluous. Nevertheless, as will be shown later, even in the case of the

Manchuria and Oderbrucker barleys, the nature of the secreting surface is of importance.

As the size of the grain increases, the efficiency of the converting power becomes a vital question. The relative decrease of the surface of the epithelial layer, together with the increased percentage of starch in the endosperm, makes an adequate amount of diastase of the utmost importance, especially if it be desired to convert starch in addition to that already in the endosperm. To have the largest contact area, the embryo must completely occupy the proximal end of the grain, and the grain itself must be as broadly oval as possible in order to provide for this maximum expansion. When these conditions are fulfilled, the resulting scutellum is a broad organ, reaching well over the shoulders of the grain and resting in a shallow, saucer-shaped depression in the endosperm.

To test the accuracy of this conclusion, examinations have been made of almost every commercial barley in the world. Several of the types found are shown in Plate V, figure 2, and in Plates VI and VII. In all cases where the grains were large, the varieties recognized as superior malting barleys each possessed a scutellum of the above type. On the other hand, barleys known to be inferior for malting were invariably characterized by the other extreme of scutellum, that is, by one which did not reach out over the shoulder of the grain. Usually a slight compensation for defective breadth of scutellum was offered by its being deeply sunken in the endosperm. This addition of surface was slight, as it must be readily realized that the increase of the few degrees of surface secured could not offset the very considerable decrease of diameter. The comparative area of the spherical surface of the large scutellum is actually greater than that of the slightly elongated, small one, so that the broad, flat type of necessity presents the greater secreting surface.

There seems to be a more or less definite correlation between the shape of the scutellum and the form of the grain. A long, pointed grain is almost invariably accompanied by a narrow, deeply sunken scutellum. Of course, the fact that the proximal end was reduced would of necessity cause a like reduction of the embryo. However, even more than such inevitable restriction usually occurs. In such grains the borders of the scutellum in a moistened grain do not ordinarily protrude at the points of contact with the aleurone layer, but are even more narrow than the available space requires. In a very few types, as the Smyrna, this is not wholly true.

While the most important factor of enzymatic production is thus seen to be the surface area of the secreting organ, there is still an additional element. There may be a difference in the quality of the secreting tissue. In other words, the epithelial layer varies with reference to the character of its cells. In some barleys it is made up of short, broad cells; in others the units are long and narrow. (See

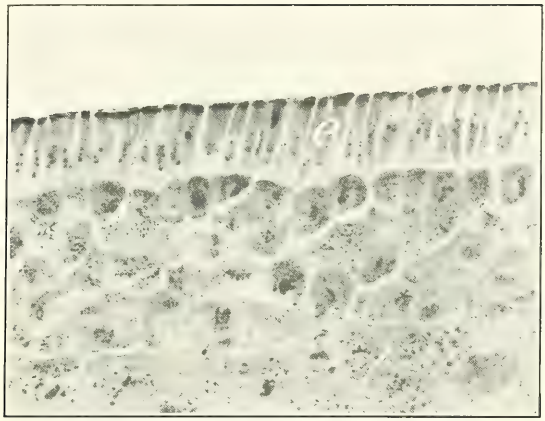


FIG. 1.—PHOTOMICROGRAPH OF THE SCUTELLUM OF A GERMINATING GRAIN WITH ITS EPITHELIAL LAYER.

In the ends of the cells of the epithelial layer (*e*) is to be seen the black deposit, thought by Torrey to be diastase. In front of the epithelial layer the endosperm has been reduced to a milky solution, while the scutellar cells back of it are obviously highly vital.

FIG. 2.—TWO GRAINS OF FINNISH BARLEY.

A grain of undesirable spindle shape and a small, insufficient scutellum.

FIG. 1.—TWO GRAINS OF PLUMAGE BARLEY.

This barley was originated by E. S. Beaven, Warminster, England. It is a grain of desirable oval shape and has a broad, ample scutellum, a combination of characters adapted to secure the highest percentage of malt extract.

FIG. 2.—TWO GRAINS OF AN ASIATIC BARLEY.

A grain of fairly oval shape, but with a small, insufficient scutellum.

fig. 6.) Given the same area in contact with the endosperm, the epithelial layer composed of long cells will have a much greater cubical content. Also, if the cells be narrower, their total number will be greater in a given area. If Torrey's conclusion that the nucleus is the final organ to which secretion is due is correct, this increase in the number of cells and in the consequent number of nuclei is an actual increase in secreting power. In any case, it is reasonable to assume that a greater number of cells with a greater plasmic content is more efficient than a smaller number of cells with a smaller plasmic content. The existence of this type of epithelial layer in the most prized malting barleys gives corroboration to this belief. The long, narrow cell prevails in the better sorts, but becomes less prominent as the quality decreases.

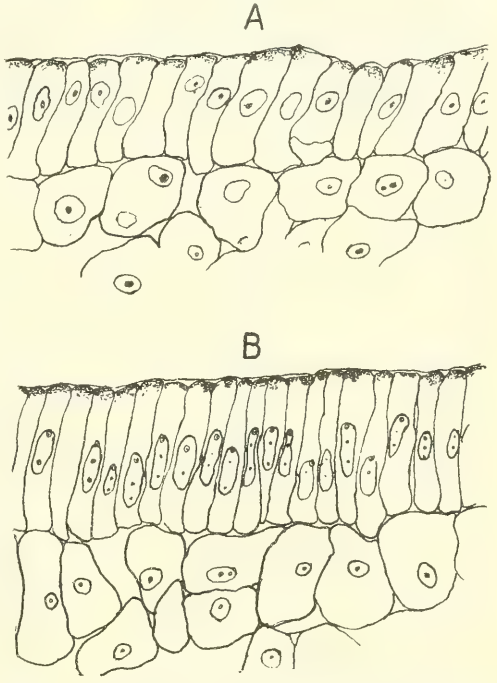


FIG. 6.—Two types of epithelial layers: *A*, An inferior epithelial layer of short, broad cells; *B*, an efficient epithelial layer of long, narrow cells. (Camera lucida drawings of actual specimens.)

An ideal grain of barley, then, is one possessing a relatively short longitudinal and a correspondingly long transverse diameter, with both the distal and proximal ends broadly oval. It contains an embryo with a large scutellum reaching over the edges of the endosperm and an epithelial layer composed of long, narrow cells.

An inferior grain of barley is elongated and is pointed at both ends. It contains an embryo with a narrow scutellum the epithelial layer of which is made up of short, broad cells.

A barley having the most perfect construction for the production of enzyms may still fall short of its highest efficiency. The size and the quality of the secreting surface are the structural factors of potential energy, but they are limited by a third element, namely, condition. If they are not in the highest state of vital energy, their maximum efficiency will not be realized; and, furthermore, this vital energy must be in a certain state of activity not entirely understood and difficult to define, which may be designated, for want of a better term, as "potency." For example, at the time of the maturity of the

grain the protoplasm of the cells of the embryo is active and possessed of all the vitality of newly formed tissue, yet barley germinates very imperfectly at this time. The epithelial layer will attack the endosperm, even under the most favorable conditions of moisture, temperature, etc., only in a weak, erratic manner. The cells do not seem ready to exert their full force. In fact, it is only after a certain period of rest that the grain displays its full germinative potency and becomes thoroughly responsive to germinative influences.

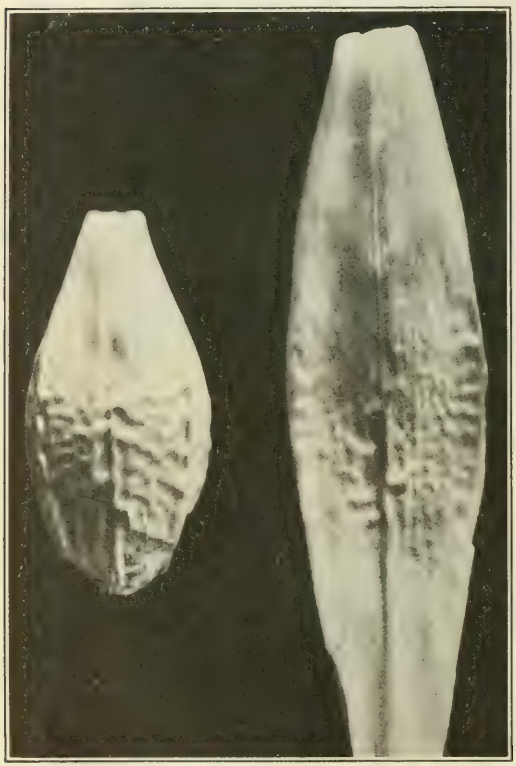


FIG. 7.—Two grains of barley from a commercial sample, showing the extremes of variation to be found in barleys as grown at present in this country. Economical malting of a barley that contains any considerable percentage of such extreme shapes is impossible.

It has been noticed that the "sweating" process in the curing of barley is closely connected with this change in the internal energy of the grain. If grain be sweated in the stack, both the percentage and the vigor of its germination are increased to the maximum. Any other method of treatment after cutting is inferior, the changes being brought about under more adverse conditions. If the curing process takes place in the shock, it occurs irregularly and imperfectly and at the same time the grain is exposed to the weather. If it occurs in the bin, there is danger of overheating and consequent damage. The vigor of germination may be affected in this way even when there is no visible indication that deterioration has occurred. Of course the injury is likely to be more serious and even to make the use of the barley for malting purposes impossible.

These various factors of efficiency in the conversion of the barley grain, when applied to commercial malting operations, presuppose pedigreed barley. The efficiency of the ordinary market barley may be increased by careful culture, but it will never become really superior. The commercial varieties as they are sold in the markets are composed of many subvarieties (fig. 7). The Manchuria, the Oder-

brucker, and the Bay Brewing usually represent mere group names for general types of barley that are in no sense pure varieties. From each of them many widely different strains may be isolated. Some of these are early and some late, some are large berried and some small berried, some germinate quickly and some slowly. When such a mixture is malted, it is obvious that the malt must be lacking in uniformity. Some of the acrospires will be protruding and some barely started. Some of the grain will be overmalted and some undermalted. The percentage of extract must necessarily be much lower than might be procured from any one element of the mixture if the elements were separated.

The purifying of the common commercial varieties will result in a more uniform malt and a higher percentage of extract. Any further step toward a greater efficiency must fall back on the use of 2-rowed barley. For botanical reasons, a 6-rowed variety can never be made as uniform in the size and character of its grain as a 2-rowed. Certain usages in manufacturing not germane to this discussion may demand 6-rowed varieties, but, considered only from the standpoint of yield of extract, the 2-rowed sorts will always remain superior.

AMERICAN BARLEYS.

The application of the factors of endosperm conversion to American conditions is more simple than the diversity of production and demand might indicate. There are in the United States but three main barley-producing areas, the Pacific coast region, the Rocky Mountain irrigated sections, and the upper Mississippi Valley. In the three Western States, California, Oregon, and Washington, the climatic conditions are such that a barley peculiar to that section is produced. The dry, sunny ripening season results in a starchy grain of very low nitrogen content. This is true regardless of variety, though variety exercises a noticeable influence. As a whole, all varieties of this district are large grained. Even the common California barley, which is 6-rowed, possesses a grain of greater size than many of the 2-rowed varieties grown elsewhere. Indeed, even when grown in the Plains States it maintains its relatively high thousand-berry weight. Regardless of variety, the problem in the West is, therefore, to secure all the enzymatic development possible. The large amount of starch endosperm to be converted makes this desirable. Although any grain possesses enough diastatic power for the conversion of its own endosperm, for malting purposes an excess is useful even though it is not used for the conversion of additional starch. A more uniform malt can be made by holding back a powerful diastase that evenly floods the endosperm than by the action of a lesser quantity that must pass from cell to cell in a more fitful attack.

In the Rocky Mountain area, the starch formation is usually not so pronounced as nearer the coast. The secreting surfaces in most vari-

eties are satisfactory, even to the extent of supplying surplus malting energy.

The upper Mississippi Valley is a section of high-nitrogen barleys. For the most part the crop is of the small-berried 6-rowed Manchuria or Oderbrucker type. It is in this region that the greatest divergence of demand is felt. A majority of the malt consumers of the Northwest use malt adjuncts. Indeed, this custom may be said for all intents and purposes to be universal. They demand a barley of high diastatic power, so as to convert the various forms of grits added to the normal starch endosperm of the malt. This demand is not in conflict with the facts of scutellar secretion. The diastatic power of small-berried barleys is simply a matter of the shape and size of the scutellum. If this power can be increased in any barley, it can also be increased in Manchuria barley. A greater diastatic power would allow the use of a grain with a larger starch endosperm and would result in a higher percentage of extract without any loss in the desired excess of diastatic energy. The Manchuria as it is sold on the Chicago markets does vary considerably in this respect. The comparison of a good-malting Manchuria with a poor-malting Manchuria barley showed that in the good barley 83 per cent of the grains had a scutellum of desirable type, while in the poor barley only 16 per cent were of this type.

Two-rowed barleys grown in the upper Mississippi Valley are likely to be much more vigorous in enzymatic action than has been thought. From all the tests of the writers these barleys are shown to be of rather high nitrogen content. This means that the grain possesses an embryo stimulated to a growth such as would provide diastase for an exceptionally large endosperm. It also means that such an endosperm has not been developed, that much of the space in the starch cells is occupied by proteid contents, and that these cells will be comparatively easily broken down. Such being the case, it is more than probable that 2-rowed barleys grown in Wisconsin, Minnesota, Iowa, and the Dakotas will be found to be able to convert a considerable amount of added starch, if handled so as to develop their greatest efficiency.

MODIFICATIONS POSSIBLE BY CULTURE.

The scope of this bulletin does not admit of a full consideration of one subject intimately connected with barley structure, namely, the great changes produced by different methods of cultivation, for a full understanding of this subject would involve a discussion of principles of plant breeding and methods of barley farming too extensive to be herein included. But at least a brief statement of the correlation between the two is necessary at this point. It may be said in general that efforts to obtain by means of culture barleys of high enzymatic

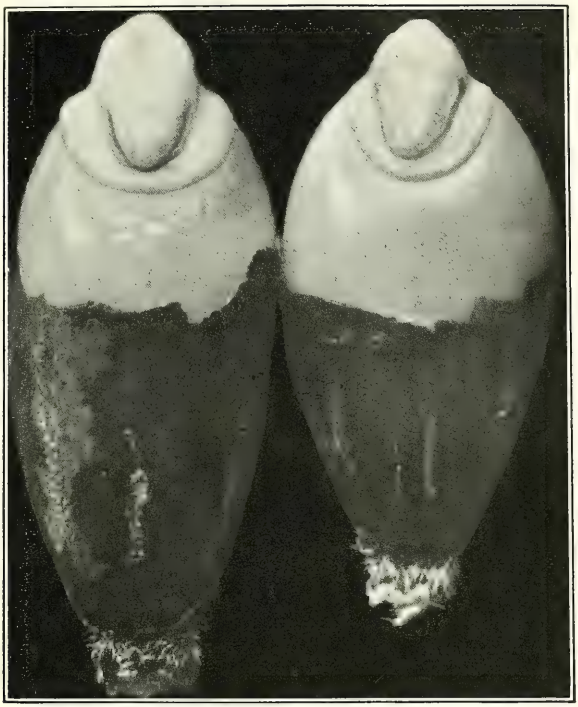


FIG. 1.—TWO GRAINS OF AN ABYSSINIAN BARLEY.

This is a fairly well-proportioned grain with a fairly ample scutellum.

FIG. 2.—TWO GRAINS OF STANDWELL BARLEY.

The broad scutellum and the thick oval grain are adapted to secure a very high percentage of malt extract.

TWO SAMPLES OF PEDIGREED PRIMUS BARLEY GROWN THE SAME SEASON AT DIFFERENT POINTS IN THE UNITED STATES, SHOWING THE EFFECT OF SOIL AND CLIMATE ON THE SCUTELLUM.

In A the scutellum has degenerated, while in B it has remained of desirable shape.

power will be most likely to succeed if they are directed separately toward the four factors of efficiency, viz: (1) The shape of the grain, (2) the size of the scutellum, (3) the quality of the epithelial layer, and (4) the vital energy of the mature grain. All of these factors but the last are varietal characters. Fortunately, varieties of extreme inferiority are for the most part already eliminated because of their low yield. The only decidedly inferior ones often met with in actual cultivation in America are certain Russian barleys with long, thin grains, a few winter varieties of similar character, and hooded sorts that probably are abnormal in form because of the lack of the functional activity of the beard.

Among the varieties the normal qualities of which are desirable, it not infrequently happens that the actual product is far from perfect; for ideal shape is largely a matter of development, even in a variety in which such is possible, and not only a matter of development but of ideal development. If the conditions of soil and cultivation are satisfactory and the climate such as will allow a correct maturation, the whole crop will consist of good, uniform seed. If these conditions do not prevail, a faulty product will result. Soil and cultivation are under the control of the farmer, at least to a limited extent, and it is the neglect of these limited influences that is responsible for much of the inferior barley produced. The English farmer has a saying that "barley is a gentleman;" that is to say, if the ideals of structure in this grain and the delicately balanced physiological functions based on structure are to be secured, barley, above all other crops, can not be treated with indifference.

The more general influences affecting adversely the cultivation of the best grades of barley by the American farmer may be briefly outlined as follows: (1) Overstimulation of purely vegetative growth by improper soil and fertilization, (2) unfavorable climatic conditions during the growing period, the factor which explains why the southern limit of good barley cultivation in the Mississippi Valley is so far north, and (3) improper methods of harvesting and curing the barley crop.

It is probable that the part of the barley grain most quickly and extensively modified by changes in the conditions of cultivation is that organ which occupies so prominent a place in these discussions—the scutellum or malting organ. Under adverse conditions or when vegetative development is unduly accelerated, the scutellum of even the best pedigreed barleys does not retain its excellence. (See Pl. VIII.) The difference between a barley having a good scutellum and one having a poor scutellum is therefore not that the good one is constant, but that under favorable conditions it and it alone will prove to be superior. Normality of development is the means of attaining

the highest success in any form, and any methods that will secure normal development will secure an ideal shape of scutellum, so far as the barley under consideration is capable of such development.

To what extent ideals in barley morphology, and especially in the form of the scutellum, may be affected by breeding, is not as yet fully known. There are many barleys which never produce a desirable malting organ. There are others which, under favorable conditions, invariably do. These facts indicate a usable varietal difference. Whether or not experiments in breeding such hopeful varieties will result in stable forms of scutellum having ideal shape and in epithelial layers having maximum enzymatic power can not at present be stated. But it is safe to assume a priori that these organs are as plastic and capable of improvement as those other parts of the barley plant, such as the shape of the head, the rigidity of the straw, etc., which have been so extensively modified by modern breeding methods. And when these morphological changes are brought about, the writers are certain that the functional qualities which these studies have demonstrated to be correlated with them will show a truly parallel improvement.

In the trials at St. Paul, Minn., in cooperation with the Minnesota Agricultural Experiment Station, a 6-rowed strain with an unusually large scutellum has been isolated from a mixed culture. As it was weak in point of yield and in strength of straw, crosses were made with it upon the Manchuria variety. The large scutellum was transmitted to the F_1 hybrids. In the F_2 generation the large scutellum was retained, but correlated with the weakness of straw and lesser yield of the better malting parent. One or two plants, however, seemed to be more intermediate in character, and if these breed true in the third generation they may form the basis for a superior strain.

FOREIGN BARLEYS.

A word should be said upon the question of the importation of foreign barleys. Barleys of superior quality are readily found abroad, and it would seem a simple matter to select the ones which most nearly satisfy American requirements and to import them for dissemination in this country. The results are, however, uniformly disappointing. The quality of such barleys is in a large part due to their adaptation to their local climatic and soil conditions. Transferred to this country they at once present other characters. In time they become acclimated, but with a few exceptions, such as the Svalof Svanhals, their performance is far from encouraging. The truly successful barleys of the future must be bred in this country. It may be that they will come from foreign stocks; indeed, there are no native sources of seed. New introductions of mixed races are, as a whole, much better material for the selection of breeding strains

than are the imported pedigreed strains. In one case there is a considerable number of subvarieties from which to choose; in the other, there is a single type that has shown superior adaptation to a widely different condition of soil and climate. In any case, whether the means be the acclimatization of established varieties or the production of new ones, the problem is not one of the country at large, but is more or less local. It is also a problem in which only the first steps of progress have been made in this country.

SUMMARY.

The integuments that envelop the ripened seed of barley arise from four sources: The nucellus, the true integuments, the walls of the ovary, and the glumes. Of these, only one has any function other than the protection afforded by dead tissue. The investing membrane of the nucellus develops into the semipermeable membrane, which is found to have remarkable selective powers.

In the development of the barley grain the endosperm develops earlier and more rapidly than the embryo, but it is the last to be completed, inasmuch as starch infiltration continues until the parent plant has ceased to live. The first starch is laid down in the center of the flanks of the grain. Infiltration of starch takes place in centrifugal order. At maturity the starch is less dense about the periphery of the endosperm than in the center. The embryo occupies a lateral position with reference to the endosperm at the proximal end of the grain. The epithelial layer is not functional until near maturity.

Germination is the continuation of the growth of the embryo which was arrested by the maturation of the seed. In its growth the embryo utilizes the food stored in the endosperm.

The conversion of the endosperm is effected by enzyms secreted by the epithelial layer of the scutellum. The cells first affected are those in contact with the epithelial layer. Conversion proceeds from the proximal end slowly toward the distal end, working more rapidly through the layers lying immediately beneath the aleurone layer. Cytase and diastase must both proceed from the scutellum, and the proteolytic ferments most probably owe their origin to the same organ.

The aleurone layer is not a secreting organ. Its function is probably mainly a protective one, although the absorption of its highly nitrogenous contents by the germinating plantlet occurs at a very opportune time in its development.

The greater diastatic power of small-berried barleys is due to the fact that the secreting area is proportionately larger. The area of the epithelial layer as a part of the surface of a spheroid must decrease less rapidly than the volume of the endosperm as the size of the barley grain is lessened.

The efficiency of conversion depends upon the shape and composition of the grain and upon the relative quantity of diastase secreted. The quantity of diastase in turn is dependent upon the size, vigor, and condition of the epithelial layer. The greatest secreting area for a given grain is secured with a scutellum extending well over the edges of the adjacent endosperm, the greatest vigor in an epithelial layer of long, narrow cells, the highest condition of efficiency in a well-matured, well-cured grain.

The ideal grain of barley is one that is broadly oval with a scutellum of the type described. If a large yield of malt extract is desired, the size of the grain should be large; if diastase be the main consideration, the size should be smaller.

Barley grains with pointed ends and a narrow scutellum are to be avoided. Poorly matured grain should also be avoided.

The highest type of the barley grain is secured only when climate, culture, and variety are all favorable.

Pedigreed varieties are essential for securing barleys of superior morphological and physiological quality. Such varieties must for the most part be produced in this country, as imported pedigreed stocks are seldom satisfactory.

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